

Effects of Veratrine on Isolated Rat Atria

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ABSTRACT

Recent literature suggests that electro-stimulation of the left atrium of rats can be potentiated by veratrine. In the present study, the effects of veratrine on spontaneous contraction of the isolated right and left atria were investigated.

The results showed that veratrine increased spontaneous contractions of the isolated right atrium of rats. At a concentration of 1×10^{-5} g/mL, veratrine caused irregular contractions of the right atrium. At a concentration of 1×10^{-4} g/mL, tetanic contractures were evoked. Verapamil and TTX inhibited the potentiation caused by veratrine. In addition, verapamil, TTX and quinidine inhibited tetanic contractures.

Veratrine potentiated contractions of the left atrium isolated from rats with or without electro-stimulation. At a concentration of 1×10^{-4} g/mL, veratrine evoked tetanic contractures of the left atrium. Verapamil, TTX and quinidine all delayed the onset of left atrial contraction and inhibited tetanic contractures.

We conclude that the tetanic contractures evoked by veratrine may be mediated by stimulation of Na^+ channels which in turn raises the level of intracellular Ca^{2+} ions.

Key words: Veratrine; Isolated rat atria; Tetanic contracture.

INTRODUCTION

Liliu (*Veratrum nigrum* L.) is a rhizocarpe of the Liliaceae family. Its main component is veratrine, which can affect excitable membranes, peripheral nerves, as well as skeletal and cardiac muscles.¹ Intravenous injection of veratrine results in hypotension and bradycardia.^{2,3} It has a positive inotropic effect on cardiac muscles and can prolong the duration

of the action potential. As a result, it depolarizes cardiac cells and delays repolarization, causing prolonged and continuous after potential. This effect is related to increased intercellular Na^+ , which in turn causes increased intracellular Ca^{2+} .⁴⁻¹⁰ Previous studies employing electro-stimulation to induce contraction in the left atrium found that veratrine increased contraction and tetanic contracture.^{11,12} The present study was based on the phenomenon of spontaneous contraction of the right atrium and observations that veratrine directly induced contraction of the left atrium.

MATERIALS AND METHODS

Atrium Preparation

Wistar rats weighing 250-300 g were struck on the back of the head before opening the chest to obtain the heart. All hearts were placed in a petri dish containing Krebs Ringer solution with 95 % O_2 and 5 % CO_2 (mM: NaCl, 118; NaHCO_3 , 25; Glucose, 11; KH_2PO_4 , 1.2; KCl, 4.75; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 1.2; $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 2.0). The atrium was surgically removed and the adipose tissue was isolated and tied to the atrium with a thread. The tip of the atrium was dipped into a Magnus organ bath containing 5 mL Krebs Ringer solution with 95 % O_2 and 5 % CO_2 . The temperature in the organ bath was maintained at $37 \pm 0.5^\circ\text{C}$. The amplitude and frequency of the right atrium contractions were recorded on a Gould polygraph through a Grass FT03 force-displacement transducer. Baseline tension of the atrium was maintained at 1.0 g. During this period, Krebs Ringer solution was freshly exchanged every 10 minutes. When the pace of contractions stabilized (frequency of contraction over 10 minutes was $\text{mean} \pm 5$ beats/minute), various antagonists were examined for their effects of the contractions evoked by veratrine.

Electro-stimulation (voltage: 0.1 V, duration: 0.8 ms, frequency: 2 Hz) was performed by connecting platinum electrodes to an electronic stimulator (Nihon Kohden MSE-3R).

Effects of Veratrine on Spontaneous Contraction of the Isolated Right Atrium

The effects of a single-concentration of veratrine on the isolated right atrium were studied. The amplitude and frequency of the atrium contractions were recorded for 10 minutes. After various concentrations (the final concentration in the organ bath: 1×10^{-8} g/mL $\sim 1 \times 10^{-4}$ g/mL) of veratrine were supplemented, the amplitude and frequency of the contractions were recorded at two minutes, and then at 5 minute intervals for 30 minutes.

The amplitudes and frequencies of the contractions to cumulative concentrations (1×10^{-8} g/mL $\sim 1 \times 10^{-4}$ g/mL) of veratrine given at 10 minute intervals were also recorded.

Various concentrations of verapamil (2.2×10^{-6} g/mL, 3.3×10^{-6} g/mL, 4.9×10^{-6} g/mL, 7.4×10^{-6} g/mL) and TTX (3.2×10^{-8} g/mL, 9.6×10^{-8} g/mL, 1.7×10^{-7} g/mL, 2.9×10^{-7} g/mL) were given 10 minutes prior to the addition of veratrine (1×10^{-5} g/mL). The effects of verapamil and TTX on the veratrine-induced contractions were recorded.

Ten minutes prior to the supplementation of veratrine (1×10^{-4} g/mL), the Krebs Ringer solution was replaced with a calcium-free Ringer solution containing 0.1 mM EGTA, 4.9×10^{-6} g/mL verapamil, 3.2×10^{-6} g/mL TTX or 7.5×10^{-5} g/mL Quinidine. The effects on the veratrine-induced tetanic contracture of the right atrium were observed and recorded.

Effects of Veratrine on the Isolated Left Atrium

Various concentrations (the final concentration of the organ bath: 1×10^{-6} g/mL $\sim$ 1×10^{-4} g/mL) of veratrine were used and the amplitude and frequency of the contractions were recorded at two minutes, and then at 5 minute intervals for 30 minutes.

Veratrine was cumulatively administrated at 10 minute intervals with or without electric stimulation. Final concentrations in the organ bath were 1×10^{-8} g/mL $\sim$ 1×10^{-4} g/mL. The amplitude and frequency of contractions were recorded after 2, 5, and 10 min.

Various concentrations of verapamil (2.2×10^{-6} g/mL, 3.3×10^{-6} g/mL, 4.9×10^{-6} g/mL and 7.4×10^{-6} g/mL), TTX (3.2×10^{-8} g/mL, 9.6×10^{-8} g/mL, 1.9×10^{-7} g/mL, 2.9×10^{-7} g/mL) and quinidine (7.5×10^{-6} g/mL, 2.3×10^{-5} g/mL, 4.5×10^{-5} g/mL, 7.5×10^{-5} g/mL) were given 10 minutes prior to the addition of veratrine (1×10^{-5} g/mL). The effects on the irregular contractures of the left atrium induced by veratrine were recorded.

10 minutes prior to adding veratrine (1×10^{-4} g/mL), the Krebs Ringer solution was replaced with a calcium-free Ringer solution containing 0.1 mM EGTA, 4.9×10^{-6} g/mL verapamil, 3.2×10^{-6} g/mL TTX or 7.5×10^{-5} g/mL quinidine. Veratrine-induced tetanic contracture of the left atrium were observed and recorded.

Drugs

Tetrodotoxin(TTX) and quinidine (RBI, USA); veratrine HCl, verapamil HCl, ethylene glycol-bis (β -aminoethyl ether) *N,N,N',N'*-tetraacetic acid (EGTA), (Sigma, USA) were dissolved in normal saline.

Statistical Analysis

The results were expressed as means $\pm$ S.E.. The differences between mean values were compared by the Student's *t* test and were considered statistically significant when $P < 0.05$.

RESULTS

Effects on the Isolated Right Atrium

The effects of various concentrations of veratrine on the contraction of the isolated right atrium of rat are showed in Fig. 1. As the concentration of veratrine increased (1×10^{-8}

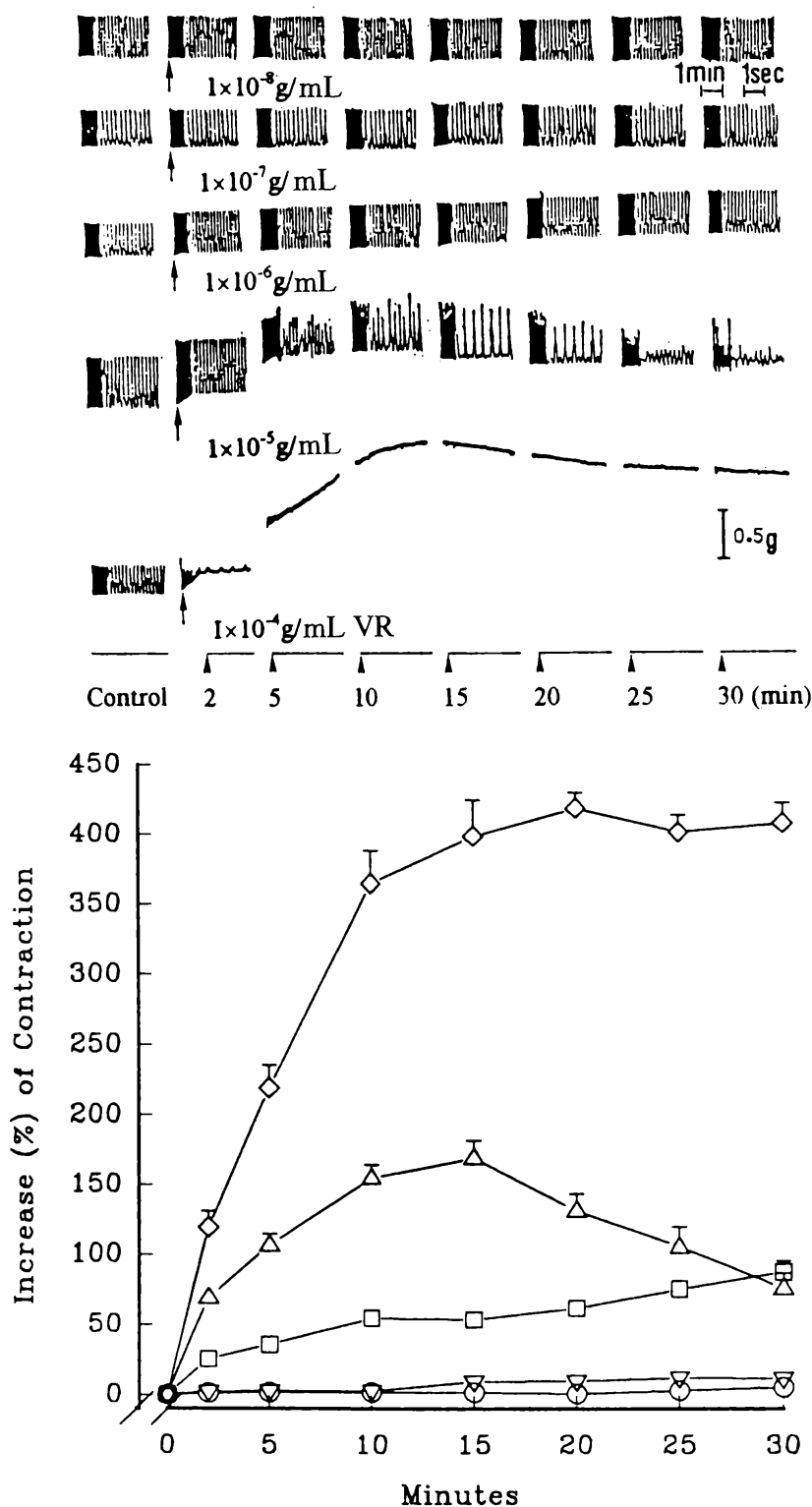


Fig. 1. The contractures induced by veratrine in isolated rat atria. O: 1×10^{-8} g/mL, ∇ : 1×10^{-7} g/mL, $\square$: 1×10^{-6} g/mL, Δ : 1×10^{-5} g/mL, $\diamond$: 1×10^{-4} g/mL. Data are shown as mean $\pm$ S.E.

g/mL $\sim 1 \times 10^{-4}$ g/mL), a gradual increase in the amplitude of the contractions was observed. The peak of the amplitude was at 15 minutes. When 1×10^{-5} g/mL of veratrine was used, irregular contractions were noted. When 1×10^{-4} g/mL of veratrine was used, tetanic contractures occurred. The effect of veratrine on the right atrium was concentration-dependent with a maximum effect at 15 minutes. Contraction frequency was positively correlated with veratrine concentration (1×10^{-8} g/mL $\sim 1 \times 10^{-6}$ g/mL) during the first 15 minutes. Thus, there was also a concentration-dependent effect (Fig. 2). However, at a veratrine concentration of 1×10^{-5} g/mL, irregular contractions were followed by a decrease in the frequency of contractions after 5 minutes.

The effects of various cumulative concentrations of veratrine on the contractions of isolated right atria are showed in Fig. 3. As veratrine concentration increased (1×10^{-8} g/mL $\sim 1 \times 10^{-4}$ g/mL), a gradual increase in contraction was observed. At a concentration of 1×10^{-5} g/mL, veratrine caused irregular contractions. Furthermore, at a concentration of 1×10^{-4} g/mL veratrine, tetanic contratures occurred. There was a concentration- and time-dependent effect on the amplitude of atrium contraction (Fig. 3A). As the concentration of veratrine increased, the frequency of the contractions decreased (Fig. 3B). Inhibition of contraction frequency increased over time.

Veratrine at a concentration of 1×10^{-5} g/mL induced irregular contractions of the right atrium. Verapamil (2.2×10^{-6} g/mL $\sim 7.4 \times 10^{-6}$ g/mL) completely inhibited the contractions

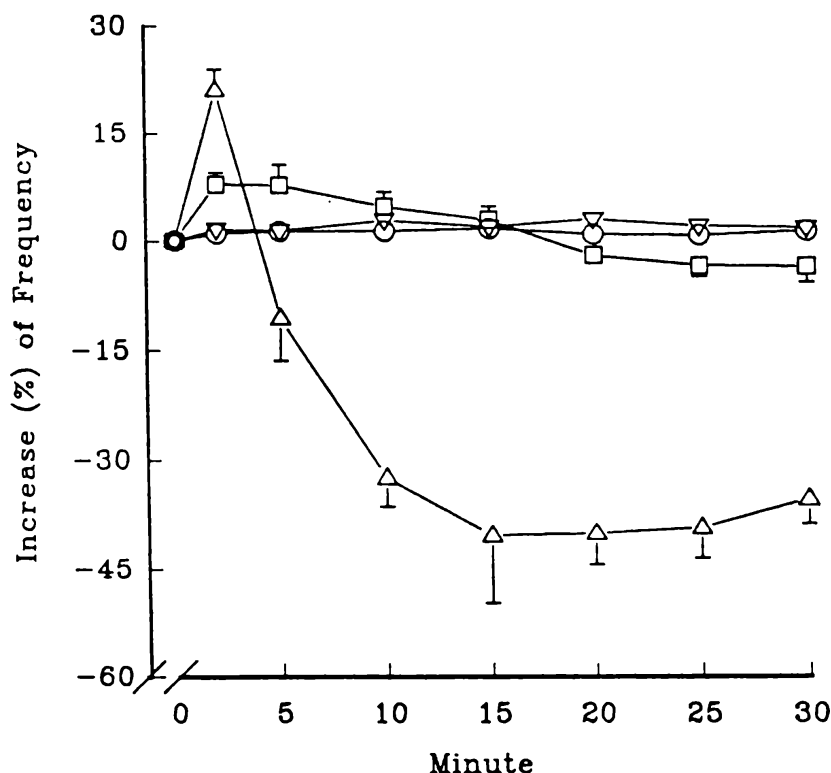


Fig. 2. The frequency contractions induced by veratrine in isolated rat atria. O: 1×10^{-8} g/mL, ▽: 1×10^{-7} g/mL, □: 1×10^{-6} g/mL, Δ: 1×10^{-5} g/mL. Data are shown as mean $\pm$ S.E.

caused by veratrine after 20 ~ 25 minutes (Fig. 4).

The effects of different concentrations of tetrodotoxin (TTX) (3.2×10^{-8} g/mL ~ $2.9 \times$

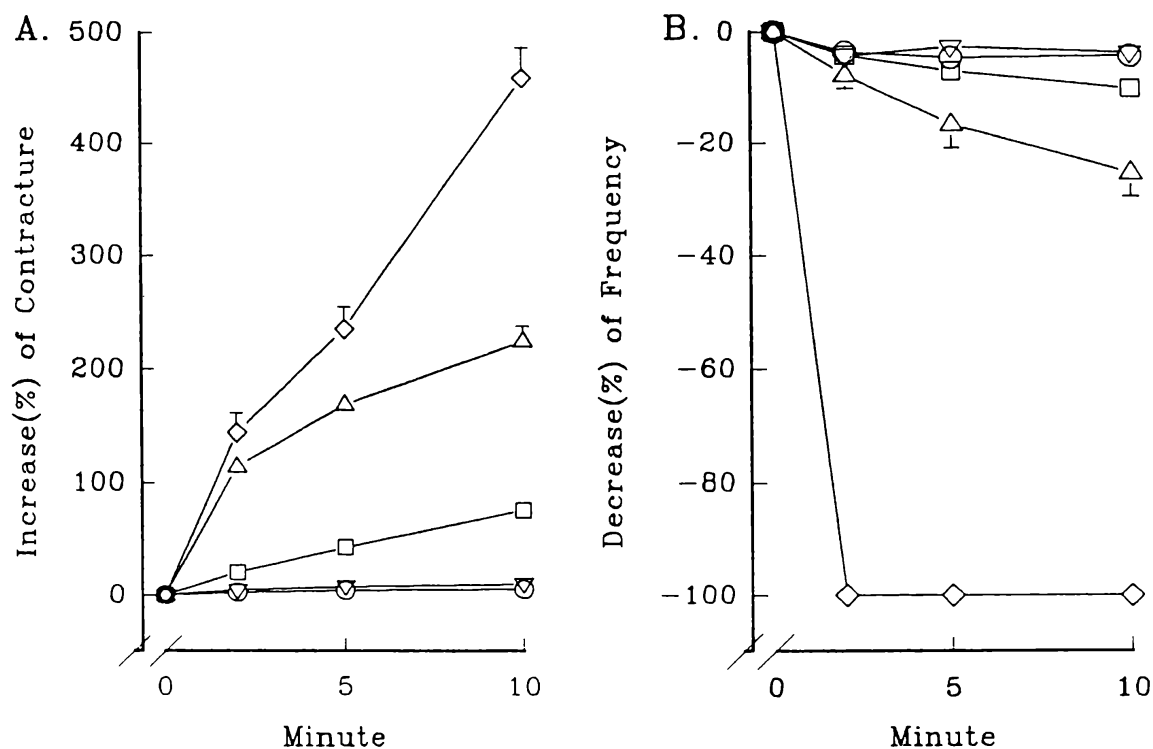


Fig. 3. The contractures induced by cumulative concentrations of veratrine in isolated rat atria. O: 1×10^{-8} g/mL, V: 1×10^{-7} g/mL, □: 1×10^{-6} g/mL, Δ: 1×10^{-5} g/mL, ◇: 1×10^{-4} g/mL. Data are shown as mean $\pm$ S.E.

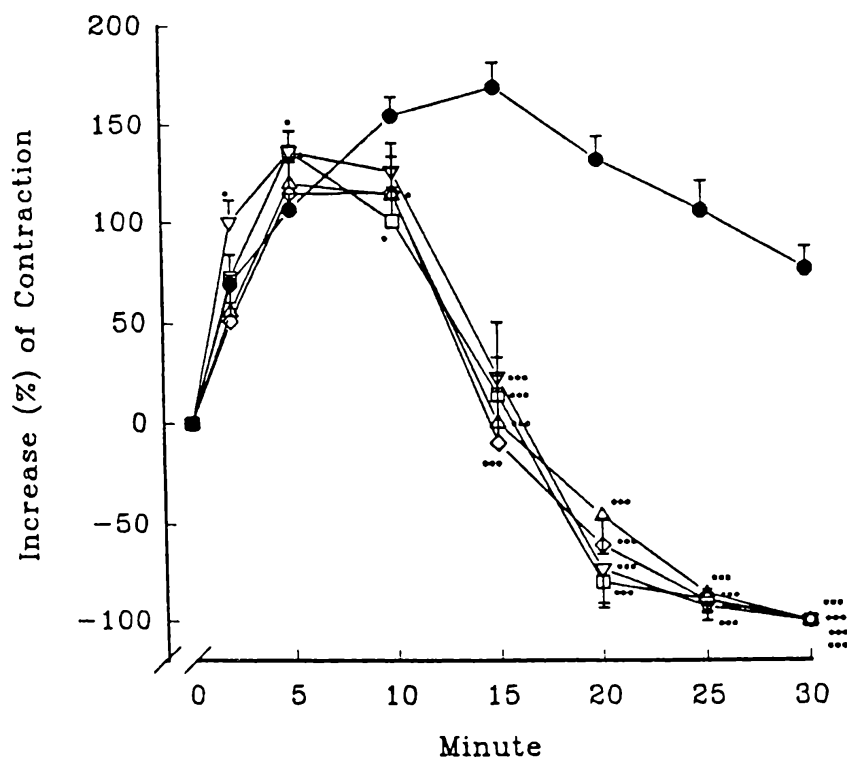


Fig. 4. The effects of verapamil (VP) on the veratrine-induced contractures of isolated rat atria. ●: Control (1×10^{-5} g/mL veratrine), V: 2.2×10^{-6} g/mL VP, □: 3.3×10^{-6} g/mL VP, Δ: 4.9×10^{-6} g/mL VP, ◇: 7.4×10^{-6} g/mL VP. Data are shown as mean $\pm$ S.E. * P < 0.05, *** P < 0.001, compared with control.

10^{-7} g/mL) are shown in Fig. 5. As the concentration of TTX increased, the frequency of the contractions induced by veratrine in the right atrium decreased with completely suppression achieved after 15 ~ 20 minutes.

The effects of Ca^{2+} removal, verapamil, TTX and quinidine on the 1×10^{-4} g/mL veratrine-induced tetanic contractures are shown in Table 1. Veratrine induced tetanic contractures were abolished in Ca^{2+} -free solution. Verapamil, TTX and quinidine also inhibited the veratrine-induced tetanic contractures of the right atrium (Table 1). However, quinidine

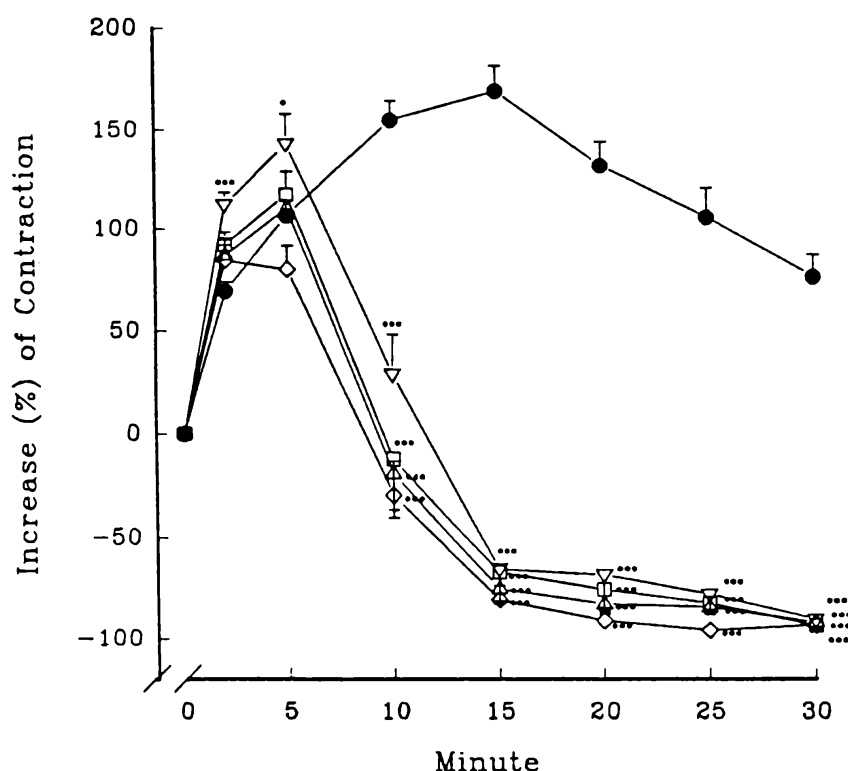


Fig. 5. The effects of tetrodotoxin (TTX) on the veratrine-induced contractures of isolated rat atria.

•: Control (1×10^{-5} g/mL veratrine), ∇ : 3.2×10^{-8} g/mL TTX, $\square$: 9.6×10^{-8} g/mL TTX, Δ : 1.9×10^{-7} g/mL TTX, $\diamond$: 7.4×10^{-6} g/mL TTX. Data are shown as mean $\pm$ S.E. * $P < 0.05$, *** $P < 0.001$, compared with control.

Table 1. The Effect of Ca^{++} -free, Verapamil (VP), Tetrodotoxin (TTX) and Quinidine (QD) on Veratrine (1×10^{-4} g/mL)-induced Tetanic Contractures in Isolated Rat Right Atria

Effect	percentage of inhibition						
	2 min	5 min	10 min	15 min	20 min	25 min	30 min
Ca^{++} -free	100 $\pm$ 0	100 $\pm$ 0	100 $\pm$ 0	100 $\pm$ 0	100 $\pm$ 0	100 $\pm$ 0	100 $\pm$ 0
Vp 4.9×10^{-6}	36.81 $\pm$ 8.36	87.94 $\pm$ 7.67	65.54 $\pm$ 13.08	42.13 $\pm$ 32.85	30.02 $\pm$ 18.84	22.86 $\pm$ 9.03	27.44 $\pm$ 10.89
TTX 3.2×10^{-6}	13.79 $\pm$ 6.72	90.51 $\pm$ 4.18	86.01 $\pm$ 6.67	69.86 $\pm$ 14.21	64.88 $\pm$ 16.71	77.84 $\pm$ 17.59	84.75 $\pm$ 8.74
QD 7.5×10^{-5}	28.27 $\pm$ 18.93	71.91 $\pm$ 11.72	86.05 $\pm$ 7.92	88.28 $\pm$ 7.97	88.96 $\pm$ 8.47	89.78 $\pm$ 8.74	90.90 $\pm$ 8.00

Data are shown as mean $\pm$ S.E..

had a reviving effect on the frequency of tetanic contractures (data not shown).

Effects on the Isolated Left Atrium

The effects of various concentrations of veratrine (1×10^{-6} g/mL $\sim$ 1×10^{-4} g/mL) on the isolated left atrium are shown in Fig. 6. Within the first two minutes, 1×10^{-5} g/mL veratrine caused continuous and irregular contractions. Tetanic contractures were observed at 1×10^{-4} g/mL veratrine. The amplitude of the contractions was concentration dependent.

Cumulative veratrine (1×10^{-6} g/mL $\sim$ 1×10^{-4} g/mL) also caused a concentration-dependent increase in contractions of the isolated left atria with electro-stimulation (Fig. 7A).

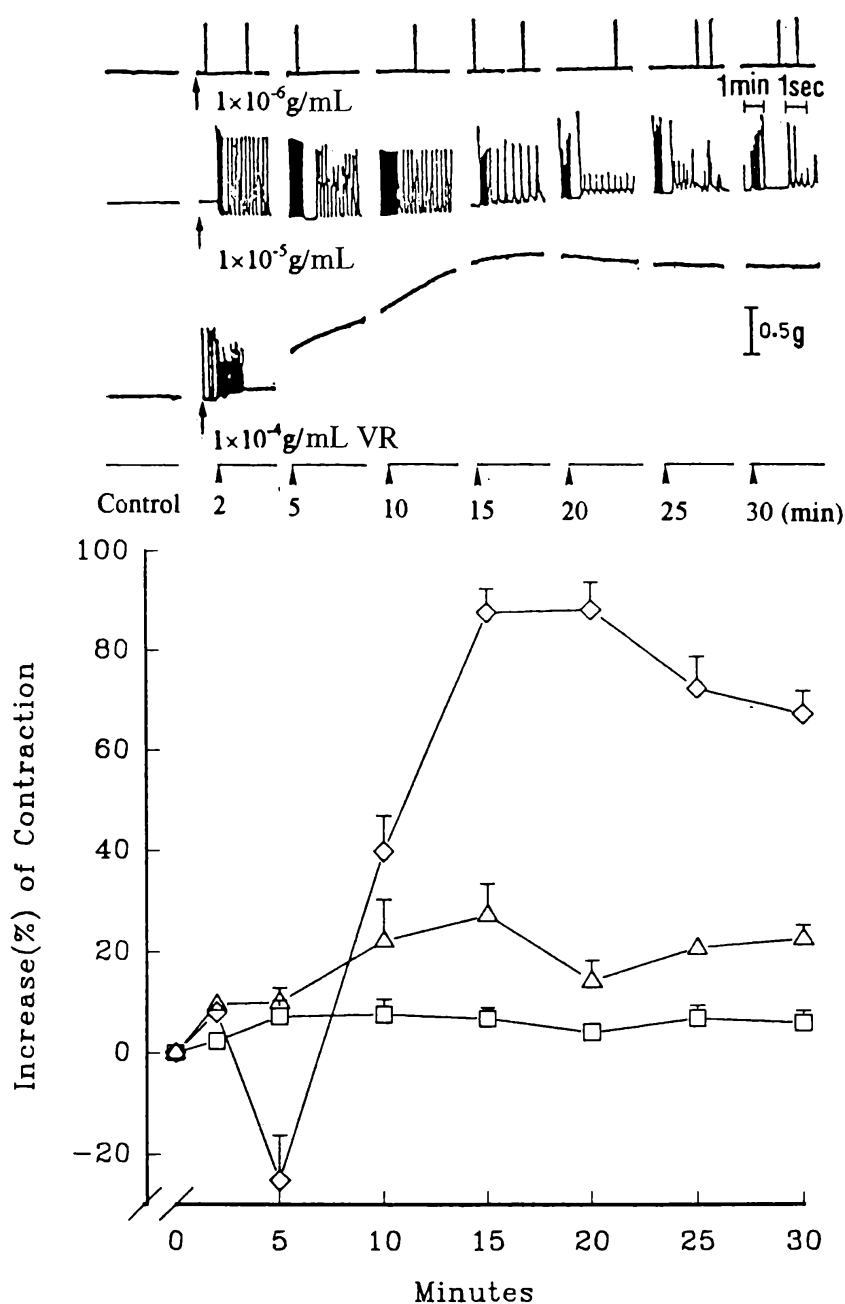


Fig. 6. The contratures induced by veratrine in isolated rat left atria. $\square$: 1×10^{-6} g/mL, Δ : 1×10^{-5} g/mL, $\diamond$: 1×10^{-4} g/mL. Data are shown as mean $\pm$ S.E.

The effects of cumulative veratrine (1×10^{-6} g/mL $\sim$ 1×10^{-4} g/mL) on the left atrium are shown in Fig. 7B. Veratrine evoked continuous contractions at 1×10^{-5} g/mL and induced tetanic contractures of the left atrium at 1×10^{-4} g/mL. The amplitude of the contractions was concentration dependent.

The effects of various concentrations of verapamil (2.2×10^{-6} g/mL $\sim$ 7.4×10^{-6} g/mL), TTX (3.2×10^{-8} g/mL $\sim$ 2.9×10^{-7} g/mL) and quinidine (7.5×10^{-6} g/mL $\sim$ 7.5×10^{-5} g/mL) on the contractions of the left atrium induced by 1×10^{-5} g/mL veratrine are shown in Table 2. Verapamil concentration-dependently delayed the onset of the contractions induced by veratrine. On the other hand, veratrine-induced contractions of the left atrium were inversely correlated with concentration (Fig. 8).

The effects of Ca^{2+} removal, verapamil, TTX, and quinidine on the tetanic contractures induced by 1×10^{-4} g/mL veratrine shown in Table 3. The veratrine-induced contractures were completely inhibited in Ca^{2+} -free solution. Verapamil, TTX and quinidine also had inhibited the veratrine-induced tetanic contractures of the left atrium.

DISCUSSION

Our results show that veratrine, Liliu's main component, increased the strength of the contractures of the rat atrium and induced irregular and tetanic contractures at concentra-

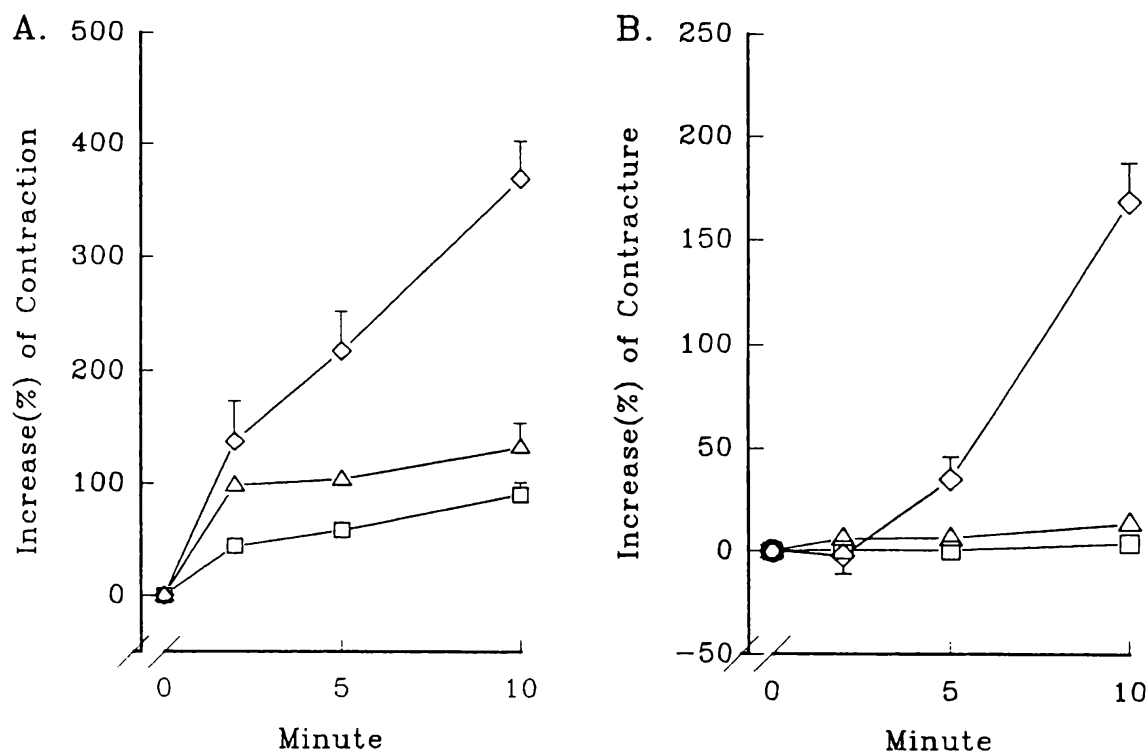


Fig. 7. The contractures induced by cumulative concentrations of veratrine in the isolated left atria. A: with electric stimulation, B: without electric stimulation, $\square$: 1×10^{-6} g/mL, Δ : 1×10^{-5} g/mL, $\diamond$: 1×10^{-4} g/mL. Data are shown as mean $\pm$ S.E.

Table 2. The Effects of Pretreatment with Verapamil (VP), TTX and Quinidine (QD) on the Onset of Veratrine (1×10^{-5} g/mL)-Induced Contractures in Isolated Rat Left Atria

Effect/Drugs (g/mL)	Onset of Contracture (min)
Control (Veratrine)	1.42 ± 0.24
VP 2.2×10^{-6}	$2.36 \pm 0.38^*$
3.3×10^{-6}	$3.64 \pm 0.44^{***}$
4.9×10^{-6}	$4.39 \pm 0.49^{***}$
7.4×10^{-6}	$6.75 \pm 0.44^{***}$
TTX 3.2×10^{-8}	$3.08 \pm 0.16^{***}$
9.6×10^{-8}	$3.12 \pm 0.31^{**}$
1.9×10^{-7}	$3.16 \pm 0.41^{**}$
2.9×10^{-7}	$3.50 \pm 0.44^{***}$
QD 7.5×10^{-6}	$2.97 \pm 0.64^*$
2.3×10^{-5}	$4.21 \pm 0.69^{***}$
4.5×10^{-5}	$17.45 \pm 1.13^{***}$
7.5×10^{-5}	$18.01 \pm 2.17^{***}$

Data are shown as mean $\pm$ S.E.. * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$ are compared with control.

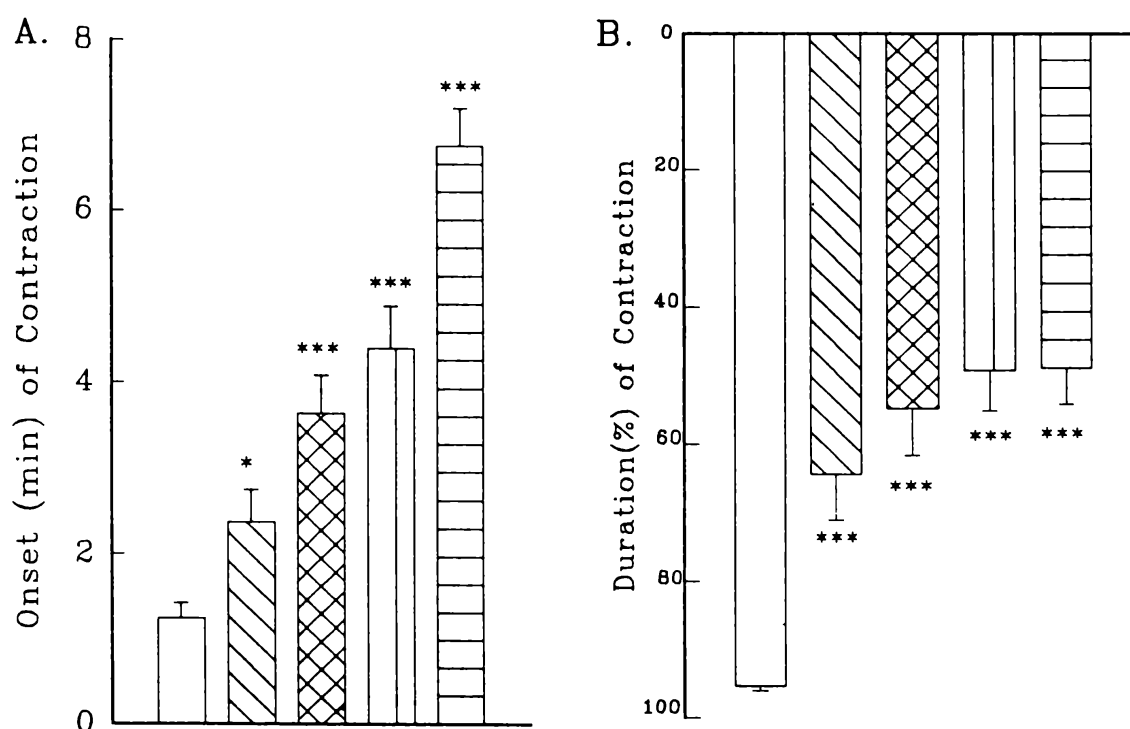


Fig. 8. The effects of verapamil (VP) on the veratrine-induced contractions in isolated left atria. A: Onset, B: Duration, □: Control (1×10^{-5} g/mL veratrine), ▨: 2.2×10^{-6} g/mL VP, ▩: 3.3×10^{-6} g/mL VP, ▤: 4.9×10^{-6} g/mL VP, ▥: 7.4×10^{-6} g/mL VP. Data are shown as mean $\pm$ S.E. * $P < 0.05$, *** $P < 0.001$, compared with control.

Table 3. The Effect of Ca^{++} -free, Verapamil (VP), Tetrodotoxin (TTX) and Quinidine (QD) on Veratrine (1×10^{-4} g/mL)-induced Tetanic Contractures in Isolated Rat Left Atria

Effect	percentage of inhibition						
Drugs(g/mL)	2 min	5 min	10 min	15 min	20 min	25 min	30 min
Ca ⁺⁺ -free	100 ± 0	100 ± 0	100 ± 0	100 ± 0	100 ± 0	100 ± 0	100 ± 0
VP 4.9 × 10 ⁻⁶	2.72 ± 1.06	81.20 ± 3.17	79.61 ± 5.12	75.92 ± 11.82	67.52 ± 14.59	62.31 ± 9.71	56.03 ± 8.86
TTX 3.2 × 10 ⁻⁶	3.55 ± 1.43	87.77 ± 3.07	92.80 ± 3.14	88.24 ± 7.93	84.45 ± 10.69	77.02 ± 14.63	73.52 ± 17.99
QD 7.5 × 10 ⁻⁵	3.33 ± 10.02	36.48 ± 10.47	74.42 ± 7.02	84.97 ± 8.30	79.94 ± 11.30	70.77 ± 13.76	70.34 ± 13.83

Data are shown as mean $\pm$ S.E..

tion levels of 1×10^{-5} g/mL and 1×10^{-4} g/mL, respectively. It was noted that veratrine caused contractures of the left atrium without electro-stimulation. Our results are similar to those of Leboeuf (1992)¹³ and Le Grand (1993)¹⁴ for electro-stimulation-induced tetanic contractures. Thus, the contractures increased with veratrine concentration.

The action of veratrine on isolated atria was inhibited by verapamil, TTX and quinidine. Verapamil is a selective L-type Ca^{2+} channel blocker¹⁵ whereas TTX is a Na^{+} channel blocker which prevents sodium influx.¹⁶ Quinidine is a class Ia antiarrhythmic agent which blocks the Na^{+} channel.¹⁷ Previous studies have shown that veratrine has a positive inotropic effect on the isolated atrium of frogs. It prolongs the action potential and its action which can be inhibited by TTX. Veratrine can increase sodium current and delay sodium inactivation to prolong sodium conductance.⁴ In addition, veratrine can affect the fast Na^{+} channels and increases sodium permeability, then opens the slow Na^{+} channels on the cardiac cells of chicken embryo. This enables Ca^{2+} influx via Ca^{2+} - Na^{+} exchange influx and thus increases the intracellular concentration of Ca^{2+} .⁶ In mammals, veratrine increases the positive inotropic action of the cardiac muscle, increasing the intracellular concentration of Na^{+} with corresponding Ca^{2+} uptake.⁷ According to the results of our experiments, the contracture of either the right or left atrium induced by veratrine could be inhibited by verapamil, TTX and quinidine. We postulate that the effects of veratrine on the atria are related to the sodium- and calcium- channels.

As veratrine-induced tetanic contractures were abolished in Ca^{2+} -free solution, the presence of Ca^{2+} ions was very important. Previous studies demonstrated that contractures caused by veratrine were influenced by extracellular Ca^{2+} concentration.¹⁴ Veratridine (1 – 10 μM) was shown to increase the Ca^{2+} -influx in the cortical synaptosomes of rat skin.¹⁸ This effect could also be inhibited by verapamil, a L-type Ca^{2+} channel blocker. In our study, the contractures induced by veratrine were markedly inhibited by verapamil. Also, TTX (1 μM) prevented veratrine (100 $\mu\text{g/mL}$)-induced tetanic contractures. If a higher

concentration (100 μM) of quinidine was used, the effects of veratrine were completely inhibited. This indicates that veratrine probably increases intracellular Na^+ concentration, which in turn increases the intracellular Ca^{2+} concentration and results in amplified atrium contractures.

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